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NOVEL NITRIC OXIDE-RELEASING AMIDINE- AND ENAMINE-DERIVED DIAZENIUMDIOLATES, COMPOSITIONS AND USES THEREOF AND METHOD OF MAKING SAME

FIELD OF THE INVENTION

The present invention relates to nitric oxide-releasing amidine- and enamine-derived diazeniumdiolates, to compositions comprising such compounds, to methods of using such compounds and compositions, to a method for the preparation of nitric oxide-releasing amidine- and enamine-derived diazeniumdiolates via the direct reaction of nitric oxide with amidines and enamines, and to a method of converting amines into such compounds.

BACKGROUND OF THE INVENTION

Nitric oxide (NO) has been implicated as part of a cascade of interacting agents involved in a wide variety of bioregulatory processes, including the physiological control of blood pressure, macrophage-induced cytostasis and cytotoxicity, and neurotransmission (Moncada et al., "Nitric Oxide from L-Arginine: A Bioregulatory System," Excerpta Medica, International Congress Series 897, Elsevier Science Publishers B.V.: Amsterdam (1990); Marletta et al., Biofactors 2: 219-225 (1990); Ignarro, Hypertension (Dallas) 16: 477-483 (1990); Kerwin et al., J. Med. Chem. 38: 4343-4362 (1995); and Anggard, Lancet 343: 1199-1206 (1994)). Given that NO plays a role in such a wide variety of bioregulatory processes, great effort has been expended to develop compounds capable of releasing NO. Some of these compounds are capable of releasing NO spontaneously, e.g., by hydrolysis in aqueous media, whereas others are capable of releasing NO upon being metabolized (Lefer et al., Drugs Future 19: 665-672 (1994)).

Keefer et al. (U.S. Patent Nos. 4,954,526; 5,039,705; 5,155,137; 5,208,233 and 5,405,919 and related patents and patent applications, all of which are incorporated herein by reference) disclose, among others,

the use of certain nucleophile/nitric oxide adducts as NO-releasing agents, i.e.,

$$\text{Nuc}-\overset{+}{\underset{\underset{O^-}{|}}{N}}=N-O^- \xrightarrow{H^+} \text{Nuc} + 2NO.$$
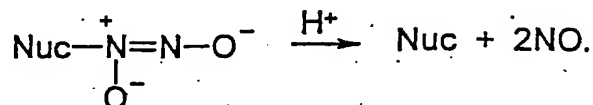
in which the nucleophilic residue (Nuc) is a primary
5 amine, a secondary amine or a polyamine. Although such
adducts offer many advantages over other currently
available nitric oxide-releasing compounds, one
disadvantage presented by the use of such adducts as
pharmaceutical agents is the potential risk of release of
10 nitrosamines, which are carcinogenic, upon decomposition
and release of NO. Another disadvantage of the adducts
of primary amines is that they can be unstable even as
solids due to a tendency to form traces of potentially
explosive diazotates.
15   Several types of compounds of the general structure

$$-\overset{\displaystyle |}{\underset{\displaystyle |}{C}}-\overset{+}{\underset{\underset{O^-}{|}}{N}}=N-OH$$
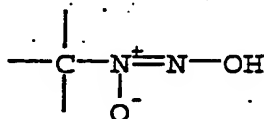
have been known for many years. Traube (<u>Liebigs Ann.
Chem.</u> <u>300</u>: 81-123 (1898)) reported the preparation of a
number of such compounds and noted that treatment of the
20 compounds with acid produced a "brown gas." Although
brown gas suggests the release of NO, given that a brown
gas also may be produced in the disproportionation of
nitrite, the release of brown gas by the compounds
prepared by Traube is not, in and of itself, evidence of
25 NO release. Compounds of the structural type reported by
Traube are known to require harsh treatment with mineral
acids to release any gas which is, of course,
incompatible with a biological utility.

Another compound, which has the structure

$$\text{Ph}-\overset{\overset{O^-}{|}}{N}=N-O^- \quad NH_4^+$$
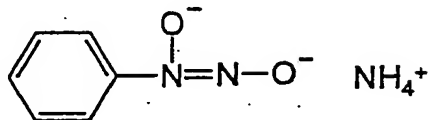
5   and which has been named cupferron, has been shown by
Kubrina et al., <u>Izvestia Akademii Nauk SSSR Seriia</u>
<u>Biologicheskaia</u> <u>6</u>: 844-850 (1988)) to generate NO *in
vivo. In addition, the antibiotics alanosine
$(C(O)(OH)CH(NH_2)CH_2 N(O)=NOH)$ and dopastin
10   $(CH_3CH=CHC(O)NHCH_2 CH(i\text{-propyl})-N(O)=NOH)$, as well as
cupferron, have been shown to release NO *in vivo* by
enzymatic oxidation (Alston et al., <u>J. Biol. Chem.</u> <u>260</u>:
4069-4074 (1985)).

     More recently, Keefer et al., in U.S. Patent No.
15   5,212,204, have broadly described that an organic moiety
may be linked via carbon to the $N_2O_2^-$ group. This patent
does not disclose an amidine or enamine structure as the
nucleophile, nor does it teach the nature of the
structural characteristics that an organic moiety must
20   possess to cause the resulting $N_2O_2^-$ group to be a nitric
oxide donor.

     Some $N_2O_2^-$-containing compounds have been disclosed
to be useful as curing agents in rubber manufacture,
antiknock additives for gasoline, indicator dyes,
25   explosives, corrosion inhibitors and fungicides (Danzig
et al., U.S. Patent No. 3,309,373; Wiersdorff et al.,
<u>Chem Abstracts 77</u>: 48034f (1972); Massengale, U.S. Patent
No. 2,635,978; and Metzger et al., U.S. Patent No.
2,954,314). However, the mechanism of the reported
30   action of these compounds was not described.

     In this regard, a recent study of the $N_2O_2^-$ group
(Taylor et al., <u>J. Org. Chem.</u> <u>60</u>: 435-444 (1995))
proposed a mechanism for the observed NO release. The
proposed mechanism was based on quantum mechanical
35   calculations which showed protonation at the terminal

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oxygen to be most favored thermodynamically in the case of N bound $N_2O_2^-$.

None of the above disclosures, however, mention anything about the release of nitroxyl (HNO, which, at the physiological pH of 7.4, exists as NO⁻) by this functional group. Recent results suggest that, under certain conditions, many classes of "NO donors" may release some NO⁻ (see the discussions for nitrosothiols and diazeniumdiolates as well as the table of NO donors in Feelisch et al., Donors of Nitrogen Oxides, In Methods in Nitric Oxide Research, M. Feelisch and J.S. Stamler, Eds., Ch. 7, pp. 71-115, John Wiley and Sons, New York (1996)).

To date, there are three compounds used to generate HNO in solution. One compound, Angeli's salt, which is the standard HNO source (Fukuto et al., J. Pharm. Exp. Ther. 263: 546-551 (1992)), is, of course, an inorganic salt. The other two compounds, acetylated Piloty's acid (Smith et al., J. Amer. Chem. Soc. 82: 5731-5740 (1960)) and benzoylated hydroxycyanamide (Lee et al., J. Med. Chem. 35 3648-3652 (1992)) are promising inhibitors of aldehyde dehydrogenase. However, even in these compounds, there is debate as to whether the observed physiological effects are attributed to NO, or to NO⁻. For example, Piloty's acid has been shown to release NO oxidatively under physiological conditions (Zamora et al., Biochem. J. 312: 333-339 (1995)).

Reports that superoxide dismutase can prolong the effects of NO via its reversible reduction to NO⁻ (Murphy et al., PNAS USA 88: 10860-10864 (1991)) and that NO⁻, itself, exhibits potent activity as a vasodilator (Fukuto et al., J. Pharm. Exp. Ther. 263: 546-551 (1992)) and as an inhibitor of aldehyde dehydrogenase (Lee et al., J. Med. Chem. 35: 3648-3652 (1992)) suggest that compounds, which release either NO or NO⁻ or mixtures of the two, are potentially useful pharmaceutical agents and may even offer advantages over compounds that just release NO.

Despite the extensive literature available on NO and
nitric oxide-releasing compounds, there remains a need
for stable nitric oxide-releasing compounds in which the
nitric oxide-releasing group $N_2O_2^-$ is bonded directly to a
carbon atom and which can be prepared from compounds that
do not include a nitrogen atom suitable for conversion to
a diazeniumdiolate.

Accordingly, it is an object of the present
invention to provide a chemical structural framework
having an atomic and electronic arrangement such that an
$N_2O_2^-$ functional group attached thereto will serve as a
spontaneous NO and/or NO⁻ donor. It is a further object
of the present invention to provide a method for
producing novel NO and/or NO⁻-releasing diazeniumdiolates
in which the N_2O_2- group is bound to a carbon atom.
Another object of the present invention is to provide NO-
and/or NO⁻-releasing derivatives of amidines and enamines.
A related object of the present invention is to provide
NO- and/or NO⁻-releasing derivatives of known
pharmaceutical agents. A more specific object is to
provide NO- and/or NO⁻-releasing derivatives of known
pharmaceutical agents whose nitrogen atoms do not provide
suitable N-diazeniumdiolates as nitric oxide donors. Yet
another object of the present invention is to provide
compositions comprising NO- and/or NO⁻-releasing
derivatives of amidines and enamines. A further object
of the present invention is to provide methods of using
NO- and/or NO⁻-releasing derivatives of amidine and
enamine compounds, and compositions thereof. These and
other objects of the present invention, as well as
additional inventive features, will be apparent from the
description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides NO- or NO⁻-releasing
diazeniumdiolates which are derived from an enamine or an
amidine and in which the $N_2O_2^-$ functional group is bonded

to a carbon atom. The present invention also provides compositions comprising such diazeniumdiolate compounds, and methods of using such compounds and compositions. The present invention further provides a method of

5 producing an NO- or NO‾-releasing enamine- or amidine-derived diazeniumdiolate. Additionally, the present invention provides a method for the preparation of an NO- and/or NO‾-releasing amidine derivative from an existing amino compound. The method comprises reaction of the

10 amino compound with an acetamidating reagent followed by reaction with nitric oxide gas.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one aspect of the invention,

15 there is provided a novel class of nitric oxide-nucleophile adducts or diazeniumdiolates having an amidine- or enamine-derived chemical linkage in which the $N_2O_2^-$ functionality is bound directly to a carbon atom of the linkage. The amidine- or enamine-derived chemical

20 linkage which includes the $N_2O_2^-$ functional group is represented by the schematic formula depicting the characteristic connectivity:

$$(C^1=)_p C^2 \sim C^3 - (N_2O_2^-)_m$$

with N^1 bonded to C^2 and $(N^2)_q$ bonded to C^3
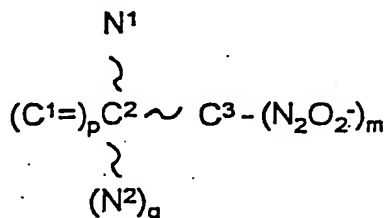
25 wherein

$C^2 \sim C^3$ means either $C^2 - C^3$ or $C^2 = C^3$

30 m is 1 or 2

q is 0 or 1

p is 0 or 1

provided that

(1) C^2 is tetravalent, and bound to two or more of C^1, C^3, N^1 and N^2;

5

(2) when p=1, then q=0 and $C^2 \sim C^3$ means C^2-C^3; or

(3) when p=0, and q=1, then $C^2 \sim C^3$ means either (i) $C^2=C^3$ or (ii) C^2-C^3 where $C^2 \sim N^1$ means $C^2=N^1$;

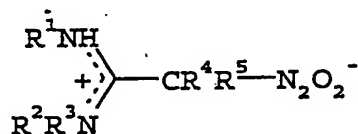(4) when $C^2 \sim C^3$ means C^2-C^3 and q=1 and p=0

10        $C^2 \sim N^1$ and $C^2 \sim N^2$ means
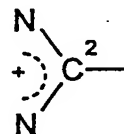
It will be appreciated by those skilled in the art that due to the nature of the synthesis reaction employed

15   as disclosed herein, the double bond in all cases would originally form as a C=N and then tautomerize if that is possible due to the presence of a C-H ß to N^1. The double bond typically tautomerizes to the more thermodynamically favored structure. However, less thermodynamically

20   favored tautomers may occur and have been observed depending on conditions such as solvent or the like. In compounds where there is no H in the β position to N^1 no tautomerization occurs. Thus, the present invention contemplates all NO-releasing diazeniumdiolates which

25   include an amidine- or an enamine-derived chemical linkage in which the $N_2O_2^-$ functional group is bound to a carbon atom irrespective of the tautomer that is thermodynamically favored. The electron movement or tautomerization for the enamines and for the amidines is

30   the same conceptually, but in the case of the enamines it is the lone pair of electrons associated with the nitrogen atom which must be used in the reaction since there is no H on the enamine nitrogen.
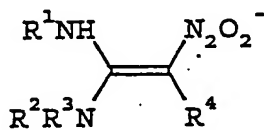
The amidine- and enamine-based diazeniumdiolates of the present invention are advantageous in several respects. These compounds are not expected to decompose to carcinogenic nitrosamines. The diazeniumdiolates of the present invention exhibit the full range of water solubility. Some of the diazeniumdiolates of the present invention are thus particularly useful where water insolubility is desirable, such as in stents, implants, prostheses and the like. Many diazeniumdiolates of the present invention are characterized by long-term slow release of NO and can be used in coatings or the like. Further, these compounds do not bleed out of the coating, even after the NO has been released. The diazeniumdiolates of the present invention are very stable solids and in solution are more heat stable than the previously described nitrogen analogs. Some can be recrystallized from boiling solvents without decomposition.

In keeping with the invention, the amidine-derived diazeniumdiolates may be further described in accordance with the following formulas:
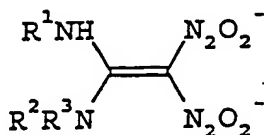
$$R^1NH \underset{R^2R^3N}{\overset{+}{>}}\!\!\!-CR^4R^5\!-N_2O_2^{-}$$

FORMULA I,

$$\underset{R^2R^3N}{R^1NH}\!\!>=<\!\!\underset{R^4}{N_2O_2^{-}}$$

FORMULA II, or

$$\underset{R^2R^3N}{R^1NH}\!\!>=<\!\!\underset{N_2O_2^{-}}{N_2O_2^{-}}$$

FORMULA III

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wherein R^1-R^5 can be a wide variety of substituents without departing from the scope of the present invention owing to the fact that any compound which includes the characteristics of the chemical linkage identified above is contemplated herein.

Thus, in the compounds of Formula I, II or III, R^1-R^3 are independently chosen from hydrogen, an unsubstituted or substituted C_{1-12} straight chain alkyl, an unsubstituted or substituted C_{3-12} branched chain alkyl, an unsubstituted or substituted C_{3-12} straight chain olefinic, an unsubstituted or substituted C_{3-12} branched chain olefinic, a substituted or unsubstituted C_{3-8} cycloalkyl, a C_{3-8} heterocyclic ring bound through a carbon atom and in which the heteroatom is oxygen or nitrogen, a substituted or unsubstituted naphthyl, a substituted or unsubstituted tetrahydronaphthyl, a substituted or unsubstituted octrahydronaphthyl, benzyl or substituted benzyl, substituted with up to three substituents, or a substituted or unsubstituted phenyl, substituted with up to three substituents.

In the compounds of Formula I, II or III, R^4 and R^5 are independently hydrogen, an unsubstituted or substituted C_{1-12} straight chain alkyl, an unsubstituted or substituted C_{3-12} branched chain alkyl, an unsubstituted or substituted C_{3-12} straight chain olefinic, an unsubstituted or substituted C_{3-12} branched chain olefinic, a substituted or unsubstituted benzyl, a substituted or unsubstituted phenyl, a substituted or unsubstituted piperazino, or a substituted or unsubstituted morpholino. R^4 and R^5 also can be amino, an unsubstituted or substituted alkylamino, carboxyalkylamino, carboxydialkylamino, an unsubstituted or substituted tolyl, xylyl, anisyl, mesityl, nitro, an unsubstituted or substituted arylamino, an unsubstituted or substituted dialkylamino, an unsubstituted or substituted diarylamino, an unsubstituted or substituted acetyl, an unsubstituted or substituted acetoxy, carboxy,

an unsubstituted or substituted carboxyalkyl, such as an
unsubstituted or substituted carboxymethyl or an
unsubstituted or substituted carboxyethyl, an
unsubstituted or substituted alkylcarbonyl, thiol, an
5   unsubstituted or substituted alkylthio, an unsubstituted
or substituted alkoxy, carboxamido, an unsubstituted or
substituted alkylcarboxamido, an unsubstituted or
substituted dialkylcarboxamido, an unsubstituted or
substituted phenoxy, an unsubstituted or substituted
10   benzyloxy, an unsubstituted or substituted nitrophenyl,
phenylcarbonyl, benzylcarbonyl, trialkylsilyl.

When any of the groups indicated above for R^1-R^5 are
identified as being substituted, such as when the C_{1-12}
straight chain alkyl, the C_{3-12} branched chain alkyl, the
15   C_{3-12} straight chain olefinic, the C_{3-12} branched chain
olefinic, the C_{3-8} cycloalkyl, the benzyl, piperazino,
morpholino, alkylamino, arylamino, acetyl, acetoxy,
carboxy, carboxymethyl, alkoxy or the like are
substituted, they can be substituted with any moiety that
20   does not destroy the NO-releasing character of the
compounds and which, preferably, is biologically
compatible. Accordingly, substituents to the substituted
R^1-R^5 groups can include hydroxy, alkoxy, acyloxy, halo or
benzyl, acetyl, carboxyl, carboxyalkyl, such as
25   carboxymethyl, carboxyethyl, carboxyalkylamido,
carboxydialkylamido, carboxamido, amino, alkylamino,
dialkylamino, alkylcarbonyl, arylamino, diarylamino,
cyano, tolyl, xylyl, mesityl, anisyl, pyrrolidinyl,
formyl, dioxane, thiol, alkylthiol, aryl, heteroaryl,
30   such as pyran, pyrrole, furan, thiophene, thiazole,
pyrazole, pyridine, or pyrimidine, phenoxy, benzyloxy,
phenylcarbonyl, benzylcarbonyl, nitrophenyl
trialkylsilyl, nitro, sulfonyl, nitrobenzyl,
trialkylammonium, alkyl, cycloalkyl, tetrahydrofuranyl,
35   tetrahydropyranyl, piperidinyl or morpholinyl.

The substituents R^1, R^2, R^3, R^4 and R^5, in various
combinations, and together with the nitrogen atom or

carbon atom to which they are bonded, can form
unsubstituted or substituted cyclic or unsubstituted or
substituted heterocyclic rings. The rings that are
formed are four member rings or layers. For example, R^1

5 and R^2 together with the nitrogen atoms to which they are
bonded can form a C_{2-8} heterocyclic ring. R^1 and R^4
together with the nitrogen atom to which R^1 is bonded and
with the carbon atom to which R^4 is bonded can form a
C_3-C_8 heterocyclic ring. Similarly, R^2 and R^3 can form a

10 C_{3-8} heterocyclic ring with the nitrogen atom to which
they are bonded. The heterocyclic ring can also include
up to one additional heteroatom, such as oxygen, nitrogen
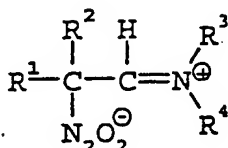or sulfur.

The heterocyclic rings formed by the different
15 combinations of R^1, R^2, R^3, R^4 and R^5 can be, for example,
a piperazino, a morpholino, a hexamethyleneimino, an
imidazolyl, a pyrrolidino, a piperidino or the like.
Likewise, R^4 and R^5 together with the carbon atom to which
they are bonded can form a C_{3-8} cycloalkyl, or a

20 heterocyclic such as tetrahydrofuranyl, dioxanyl or the
like. Further, R^4 and R^5 together with the carbon atom to
which they are bonded can form a 1,4-benzodioxane, 1,3-
benzodioxole, tetrahydronaphthlene, octahydronaphthalene,
piperazine, morpholine, tetrahydroquinoline,

25 tetrahydroquinoxaline, or tetrahydroisoquinoline.

Each of the cyclic or heterocyclic rings formed with
R^1 and R^2, or R^2 and R^3, or R^1 and R^4, or R^4 and R^5 can be
substituted with one or more substituents, including, by
way of example, C_{3-8} cycloalkyl, alkoxy, benzyl, fused

30 benzene, phenyl, an alkoxy, acetyl, carboxyl,
carboxymethyl, carboxyethyl, carboxamido, amino, alkyl
amino, dialkylamino, pyrrolidine, dioxane, thiol or
alkylthiol, or a heteroaryl such as pyran, pyrrole,
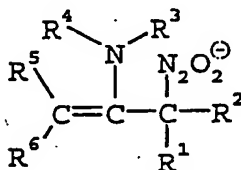furan, thiophene, thiazole, pyrazole, pyridine, or

35 pyrimidine.

The compounds of the present invention can be
derived from existing pharmaceutical agents that contain

the amidine group. For example, a compound of Formula
III preferably is one in which R^1 and R^2 are hydrogen and.
R^3 is the entire substituent attached to an amine of a
pharmaceutical agent such as, for example, tryptamine,
serotonin, histamine, valcyclovir, adenosine, thyroxine,
guanine, guanosine, ubenimex, glucosamine, mannosamine,
mycosamine, sphingosine, thienamycin, penicillamine and
rimantadine. Similarly, for example, the present
invention provides a compound of Formula III, in which R^1
and R^2 are hydrogen and R^3 is the entire substituent
attached to an amine of an amino acid. The amino acid is
preferably lysine, tryptophan or hydroxy-tryptophan.

The present invention also provides compounds of

$$R^1 - \underset{\underset{N_2O_2^{\ominus}}{|}}{\overset{\overset{R^2}{|}}{C}} - \underset{\underset{|}{|}}{\overset{\overset{H}{|}}{C}} = \overset{\oplus}{N} \underset{R^4}{\overset{R^3}{\diagdown}}$$

FORMULA IV and

$$\underset{R^6}{\overset{R^5}{\diagdown}} C = C - \underset{\underset{R^1}{|}}{\overset{\overset{N_2O_2^{\ominus}}{|}}{C}} - R^2 \qquad \underset{R^4}{\overset{R^3}{\diagup}} N$$

FORMULA V

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wherein R^1-R^6 can be a wide variety of substituents
without departing from the scope of the present invention
10 owing to the fact that any compound which includes the
characteristics of the chemical linkage identified above
is contemplated herein.

    Thus, in the compounds of Formula IV and Formula V,
R^1, R^2, R^5 and R^6 are independently hydrogen, an
15 unsubstituted or substituted C_{1-12} straight chain alkyl, an
unsubstituted or substituted C_{3-12} branched chain alkyl, an
unsubstituted or substituted C_{3-12} straight chain olefinic,
an unsubstituted or substituted C_{3-12} branched chain
olefinic, a substituted or unsubstituted benzyl, a
20 substituted or unsubstituted piperazino, a substituted or
unsubstituted morpholino, amino, an unsubstituted or
substituted alkylamino, an unsubstituted or substituted
arylamino, an unsubstituted or substituted dialkylamino,
an unsubstituted or substituted diarylamino,
25 carboxyalkylamino, carboxydialkylamino, cyano, tolyl,
xylyl, anisyl, mesityl, nitro, an unsubstituted or
substituted acetyl, an unsubstituted or substituted
acetoxy, carboxy, an unsubstituted carboxyalkyl, such as
an unsubstituted or substituted carboxymethyl, or an
30 unsubstituted or substituted carboxyethyl, an

unsubstituted or substituted alkylcarbonyl, thiol, an
unsubstituted or substituted alkylthio, an unsubstituted
or substituted alkoxy, carboxamido, an unsubstituted or
substituted alkylcarboxamido, or an unsubstituted or
5   substituted dialkylcarboxamido.

    In the compounds of Formula IV and V, R^3 and R^4 are
independently chosen from hydrogen, an unsubstituted or
substituted C_{1-12} straight chain alkyl, an unsubstituted or
substituted C_{3-12} branched chain alkyl, an unsubstituted or
10 substituted C_{3-12} straight chain olefinic, an unsubstituted
or substituted C_{3-12} branched chain olefinic, a substituted
or unsubstituted C_{3-8} cycloalkyl, a C_{3-8} heterocyclic ring
bound through a carbon atom and in which the heteroatom
is oxygen or nitrogen, a substituted or unsubstituted
15 naphthyl, a substituted or unsubstituted
tetrahydronaphthyl, a substituted or unsubstituted
octahydronaphthyl, benzyl or substituted benzyl,
substituted with up to three substituents, or a
substituted or unsubstituted phenyl, substituted with up
20 to three substituents. Such compounds are advantageous
because they are more "organic" than polyamines, such
that simple aromatic enamines can be made to be water-
insoluble, yet release NO, and to be heat-stable.

    When any of the groups indicated above for R^1-R^5 are
25 identified as being substituted, such as the C_{1-12} straight
chain alkyl, the C_{3-12} branched chain alkyl, the C_{3-12}
straight chain olefinic, the C_{3-12} branched chain olefinic,
the C_{3-8} cycloalkyl, the benzyl, piperazino, morpholino,
alkylamino, arylamino acetyl, acetoxy carboxy,
30 carboxymethyl alkoxy or the like, they can be substituted
with any moiety that does not destroy the NO-releasing
character of the compounds and which, preferably, is
biologically compatible. Accordingly, substituents to
the substituted R^1-R^5 groups can include hydroxy, alkoxy,
35 acyloxy, halo or benzyl, acetyl, carboxyl, carboxyalkyl,
such as carboxymethyl, carboxyethyl, carboxyalkylamido,
carboxydialkylamido, carboxamido, amino, alkyl amino,

dialkylamino, alkylcarbonyl, arylamino, diarylamino, tolyl, xylyl, mesityl, anisyl, pyrrolidine, formyl, dioxane, thiol, alkylthiol, aryl, heteroaryl, such as pyran, pyrrole, furan, thiophene, thiazole, pyrazole,
5 pyridine, or pyrimidine, phenoxy, benzyloxy, phenylcarbonyl, benzylcarbonyl, nitrophenyl trialkylsilyl, nitro.

The groups R^1-R^6 of the compounds of Formula IV and Formula V in various combinations, and together with the
10 nitrogen atom or carbon atom to which they are bonded and intervening atoms, can form heterocyclic rings. For example, and not in limitation, a compound of Formula V in which R^3 and R^4, together with the nitrogen atom to which they are bonded, can form a C_{3-8} heterocycle. The
15 heterocycle can be further substituted with a heteroatom. As another example, in the Formula V compound, R^1 and R^6, together with the C=C-C through which they are bonded, can form a substituted or unsubstituted C_{3-12} cycloalkyl. Similarly, for a compound of Formula IV, R^2 and R^3,
20 together with the nitrogen to which R^3 is bonded, can form a C_{3-8} heterocycle. The heterocycle can be further substituted with a heteroatom, or an aromatic ring, which can be substituted with a C_{1-6} alkyl or a C_{1-6} alkoxy. Also, R^5 and R^4 can form a C_{3-8} heterocycle, which can also
25 be substituted.

In Formulas IV and V, R^3 and R^4 together with the nitrogen atom to which they are bonded can form a C_{3-8} heterocyclic ring or a C_{3-8} substituted heterocyclic ring or a C_{3-8} unsubstituted or substituted heterocyclic ring
30 containing up to two additional heteroatoms selected from the group O, S, N.

Also, R^5 and R^6 together with the carbon to which they are bonded can form a substituted or unsubstituted C_{4-8} cycloalkyl.
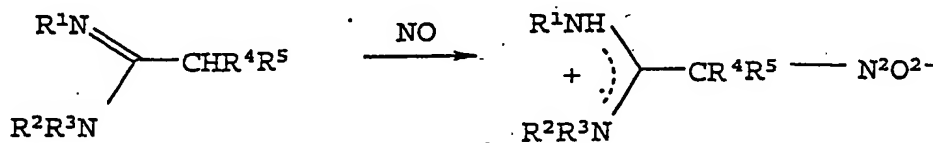35 With respect to the compounds of Formulas I, II and III, R^1-R^5 can be selected such that they represent the substituents attached to the amidine of nasal

decongestants and α-adrenergic antagonists such as
tetrahydrozoline, idazoxan, phentolamine, xylometazoline
and the like.

In accordance with another aspect of the invention,
there is provided a method for the preparation of the
amidine- and enamine-derived NO-releasing compounds
described herein. In one embodiment, the method
comprises reacting an amidine, preferably an amidine of
Formula Ia, IIa or IIIa, with gaseous NO in acetonitrile
or a similar solvent to produce an $N_2O_2^-$-containing
compound. R^1 and R^4 together with the nitrogen atom to
which R^1 is bonded and with the carbon atom to which R^4 is
bonded can form a C_3-C_8 heterocyclic ring.

The solvent is preferably chosen so that the
starting amidine or enamine is soluble whereas the
resulting $N_2O_2^-$-containing product is insoluble and so
precipitates as it forms in order to drive the reaction
to completion. Anhydrous and neutral solvents such as
acetonitrile, tetrahydrofuran, dioxane and ether are
preferred because they do not cause hydrolysis of the
water-sensitive amidines and enamines. However, it is
anticipated that low yields of the desired products can
also form in partly aqueous and/or basic solvents such as
NaOMe in methanol or wet tetrahydrofuran among others,
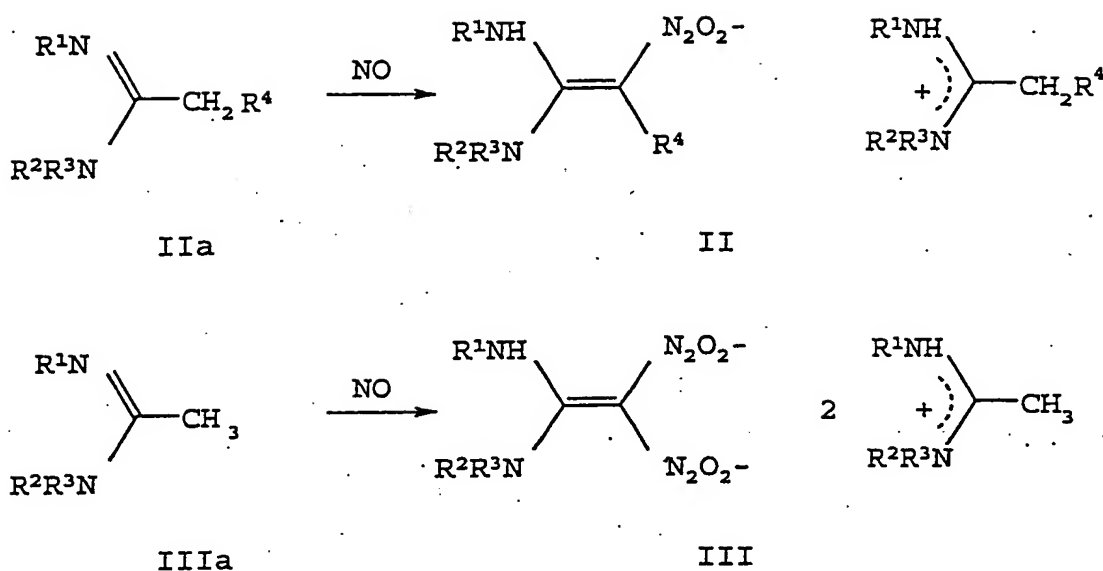and such solvents may also be used.

The resulting compound in accordance with the method
of the invention contains either one or two $N_2O_2^-$
functional groups depending upon the structure of the
amidine reactant, as, for example, shown below.

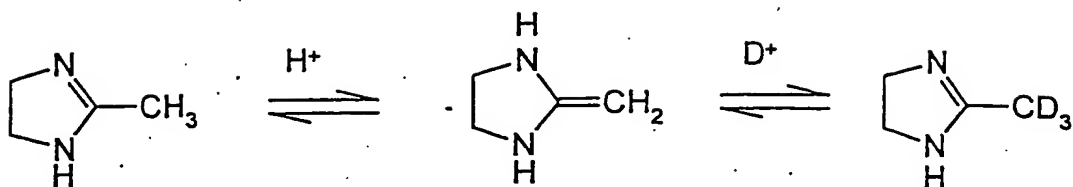$$R^1N\diagdown\diagdown\hspace{-1em}\diagup -CHR^4R^5 \xrightarrow{\ NO\ } \begin{array}{c} R^1NH \\ + \\ R^2R^3N \end{array} \diagdown\hspace{-0.5em}\diagup -CR^4R^5 \text{------} N^2O^{2-}$$

$$R^2R^3N$$

Ia   I

$$\underset{\text{IIa}}{\underset{R^2R^3N}{\overset{R^1N}{>}}C-CH_2R^4} \quad \xrightarrow{\text{NO}} \quad \underset{\text{II}}{\underset{R^2R^3N}{\overset{R^1NH}{>}}C=\overset{N_2O_2^-}{\underset{R^4}{C}}} \quad \underset{R^2R^3N}{\overset{R^1NH}{>}}\overset{+}{C}-CH_2R^4$$

$$\underset{\text{IIIa}}{\underset{R^2R^3N}{\overset{R^1N}{>}}C-CH_3} \quad \xrightarrow{\text{NO}} \quad \underset{\text{III}}{\underset{R^2R^3N}{\overset{R^1NH}{>}}C=\underset{N_2O_2^-}{\overset{N_2O_2^-}{C}}} \quad 2\; \underset{R^2R^3N}{\overset{R^1NH}{>}}\overset{+}{C}-CH_3$$

Methods for the preparation of the amidines, such as those of Formulas Ia, IIa and IIIa, are well known and have been reviewed in two reference books, Gautier et al., "Preparation and Synthetic Uses of Amidines," Chapter 7 in The Chemistry of Amidines and Imidates, Editor: Patai, pp. 283-348, Wiley, 1975, and Boyd, "Recent Advances in the Synthesis of Amidines," Chapter 7 in The Chemistry of Amidines and Imidates, Volume 2, Editors: Patai and Rappoport, pp. 339-367, Wiley, 1991. These methods can be used by those skilled in the art to prepare a wide variety of amidines which can then be made into NO-releasing diazeniumdiolates in accordance with the invention.

By way of example and not in limitation, the preparation of an NO-releasing amidine-derived diazeniumdiolate can be illustrated by the reaction of 2-methyl-2-imidazoline with NO as follows:
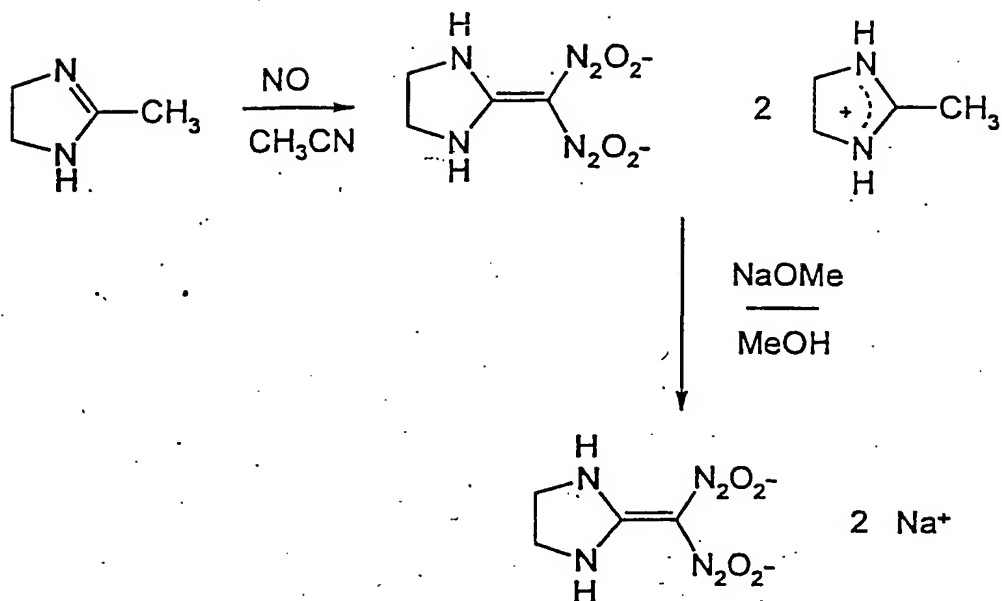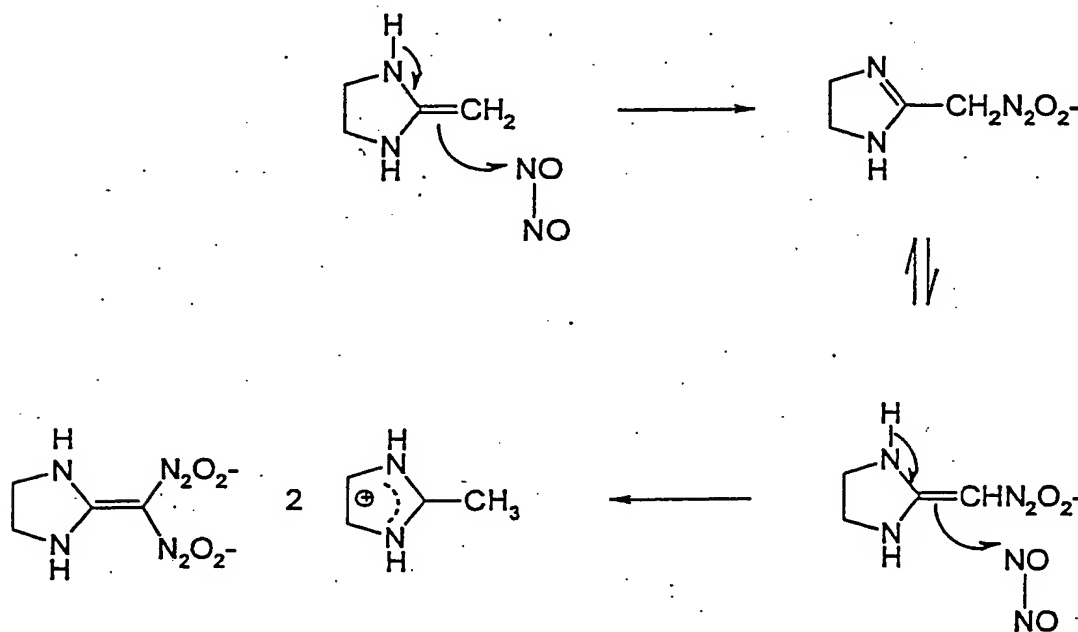
Although the initial reaction products are the amidinium salts (either intramolecular or

5   intermolecular), standard metathesis reactions can be employed to change the cation to any pharmaceutically acceptable ion. This is illustrated above by the reaction involving sodium methoxide in methanol, which produces the disodium salt. Also, by varying the

10   synthesis procedures, the intramolecular or intermolecular salt or a mixture thereof can be obtained; the reaction of 2-methyl-2-imidazoline with NO in NaOMe/MeOH to directly form the sodium salt is an example of such a reaction.

15   While applicants do not wish to be bound to any particular theory, the above reactions are believed to be explained by the reaction of NO with the little exploited enediamine tautomers of the amidines. The enediamine tautomers are known to exist in solution and were first

20   proposed to explain deuterium exchange in NMR solutions as follows (Isagulyants et al., Zh. Prikl. Khim. 41: 1585-1590 (1968); also, in Chem. Abstracts 70: 11629h (1969)):

$$\text{(2-methyl-2-imidazoline)} \quad \overset{H^+}{\rightleftharpoons} \quad \text{(exocyclic }=CH_2\text{)} \quad \overset{D^+}{\rightleftharpoons} \quad \text{(}CD_3\text{ derivative)}$$

Accordingly, while not being bound to any particular theory, it is believed that the reaction of the above undeuterated compound with an NO dimer is as follows:

$$\text{(} =CH_2 \text{ intermediate with NO dimer)} \longrightarrow \text{(}-CH_2N_2O_2^-\text{)}$$

$$\text{(}N_2O_2^- / N_2O_2^- \text{ bis-adduct)} \qquad 2 \text{ (imidazolinium)} -CH_3 \longleftarrow \text{(}=CHN_2O_2^- \text{ with NO dimer)}$$

The reaction is believed to stop at this stage due to steric hindrance and/or precipitation of the product from solution.

When either the amidinium or sodium salt of the NO-releasing diazeniumdiolate derived from 2-methyl-2-imidazoline was dissolved in water and acidified, a voluminous gas evolution resulted and the solution turned blue in color and remained so for many hours after gas evolution had ceased. When the experiment was repeated at pH 7.4, the evolving gas was identified as a mixture of 2 parts NO (determined by chemiluminescence) and 1

part N_2O (determined by gas chromatography). Nitrous oxide (N_2O), being the end product of HNO dimerization and dehydration, provided a measure of HNO production via the equation (Nagasawa et al., J. Med. Chem. 33: 3122-3124

5   (1990)):

$$2HN=O \longrightarrow \left[H{-}O{-}N{=}N{-}O{-}H \right] \longrightarrow N_2O \;+\; H_2O$$
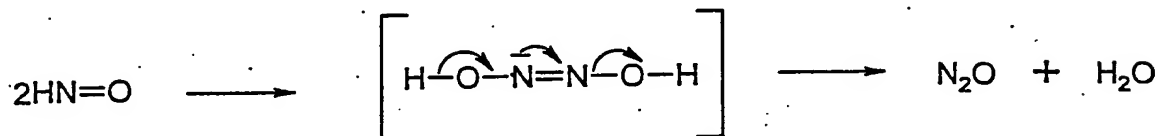
10   Again, while not wishing to be bound to any particular theory, it is believed that the partial mechanistic explanation for these observations is as follows:
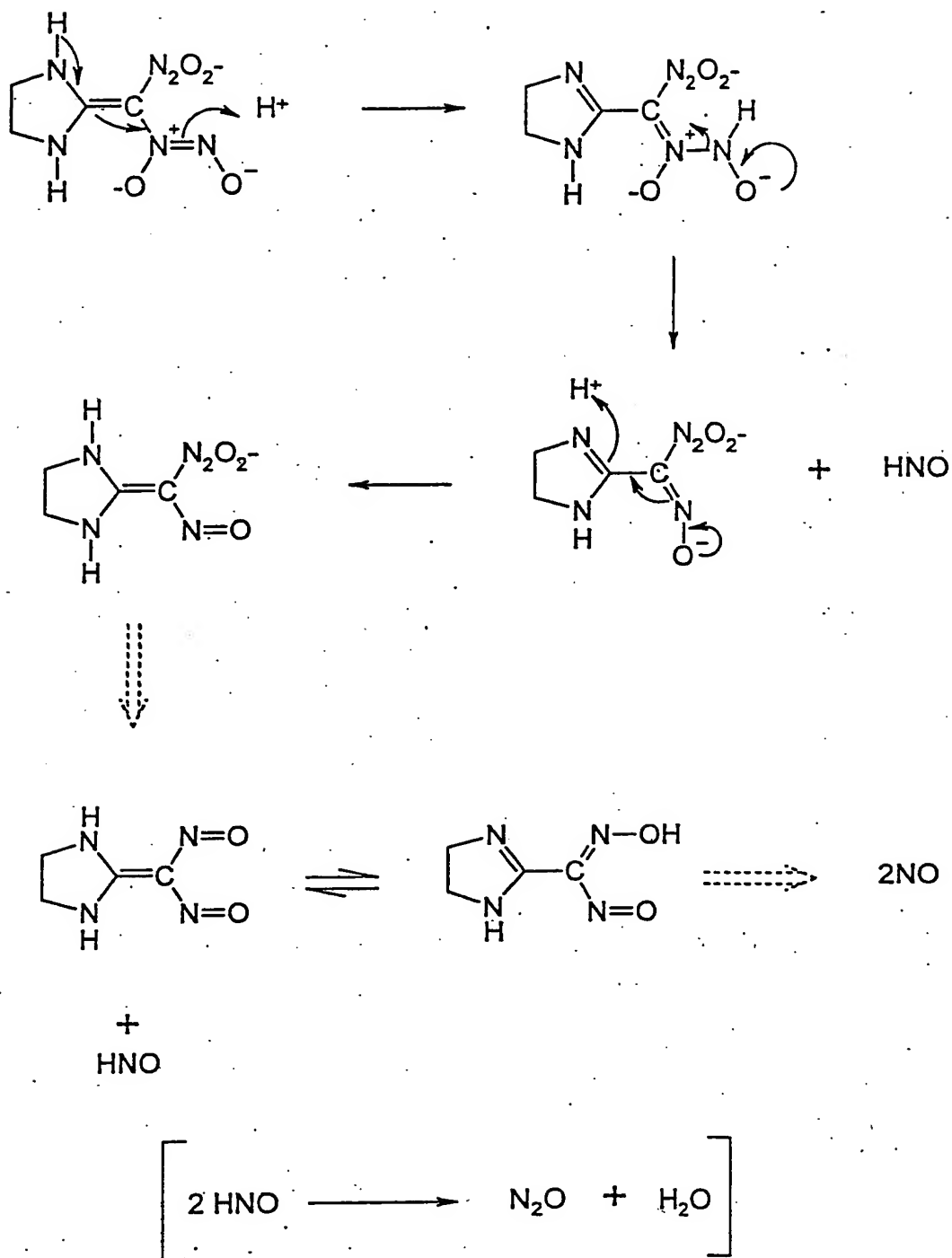
The last step in this mechanism is not well understood but has precedent in the known release of NO by FK409 and closely related compounds which are used as standard sources of NO (Kita et al., *Eur. J. Pharmacol.* 257: 123-130 (1994)). Although this mechanism is one explanation for the observed NO and N$_2$O release, it is a

very incomplete representation of what actually happens
to any given compound in aqueous solution. Specifically,
amidines are known to be subject to hydrolysis at rates
that range from very slow, such as for 2-methyl-2-

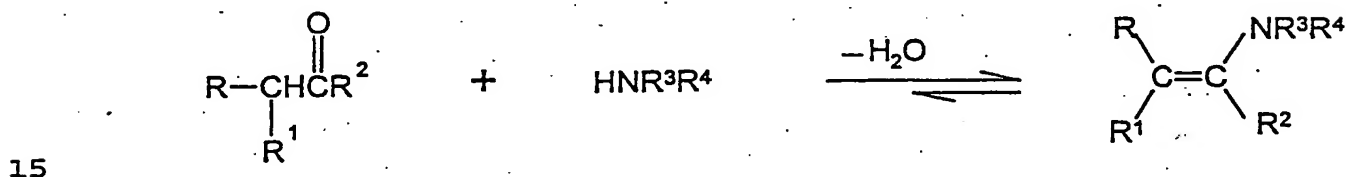5   imidazoline (Haake et al., J. Org. Chem. 35: 4063-4067
(1970))

$$CH_3 \overset{O}{\overset{\|}{C}} NHCH_2CH_2NH_2$$
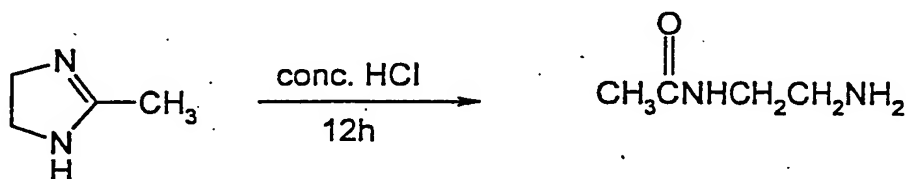
10   to very fast for acetamidine (Davies et al, Chem.
Ind. (London): 628 (1958)).

$$CH_3 \overset{O}{\overset{\|}{C}} NH_2$$
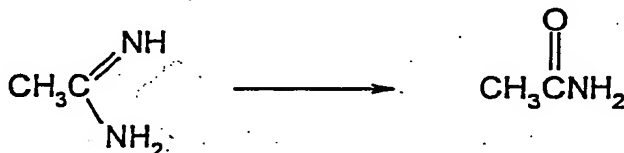
15   Thus, at any intermediate stage of HNO or NO
release, the amidino group could hydrolyze and no further
gas would be generated. A compound in which the amidine
hydrolyzes rapidly would release much HNO but very little
NO, whereas a compound in which the amidine hydrolyzes

20   slowly would have time for NO release, which is the last
step, and would thus release a larger mount of NO. In
this regard, compounds of Formula I (as set forth above)
cannot be hydrolyzed by the above mechanism. It is
believed that these mono-$N_2O_2^-$ derivatives break down via

25 · two competing pathways, one of which appears to be simple
reversal of the synthesis step to release NO, while the
other may be a single scission to yield one molecule of
HNO and a mono-C-nitroso compound. Since the amidino
tautomers cannot come into conjugation with this nitroso

30   group, it does not serve as a source of NO, and since
hydrolysis of the amidine competes with the first

pathway, compounds derived from amidines of formula I release only small amounts of NO, but over a long period of time. In such cases, the reaction of an amidine with NO results in a sterically hindered compound of formula

5 I, which is apparently inclined to break apart differently than previously reported, less hindered $N_2O_2^-$ compounds.

In another embodiment of the present inventive method, an enamine, preferably an enamine of Formula IV

10 or V, is reacted with NO to produce an $N_2O_2^-$ containing compound. Enamines are prepared from an equimolar mixture of an aldehyde or ketone and a secondary amine via dehydration as follows.

$$ R-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle R^1}{|}}{C}}HCR^2 \quad + \quad HNR^3R^4 \quad \xrightarrow[]{-H_2O} \quad \overset{R}{\underset{R^1}{C}}=\overset{NR^3R^4}{\underset{R^2}{C}} $$

15

Methods for preparing enamines and lengthy discussions of their properties are readily available to synthetic chemists (see, e.g., Hickmott, Tetrahedron 38:

20 1975-2050 (1982); Hickmott, Tetrahedron 38: 3363-3446 (1982); Cook, Enamines: Synthesis, Structure and Reactions, Marcel Dekker, New York (1988); and Szmuszkovicz, Enamines, Vol. 4 of Adv. in Org. Chem. Methods and Results, Wiley Interscience, New York

25 (1963)). Although literally thousands of carbonyl compounds are used in this reaction, the amines are usually limited to a select few, such as dimethylamine, diethylamine, piperidine, pyrrolidine, morpholine, and N-methylaniline.

30 Unlike the amidine-derived compounds, the enamine-derived diazeniumdiolates do not appear to release any NO^- or N_2O. Rather, they release small amounts of NO over prolonged periods of time (e.g., 1 week in phosphate-buffered saline). As with amidine-derived compounds, the

mechanism of NO release is complicated by a competing
hydrolysis mechanism as set forth below.
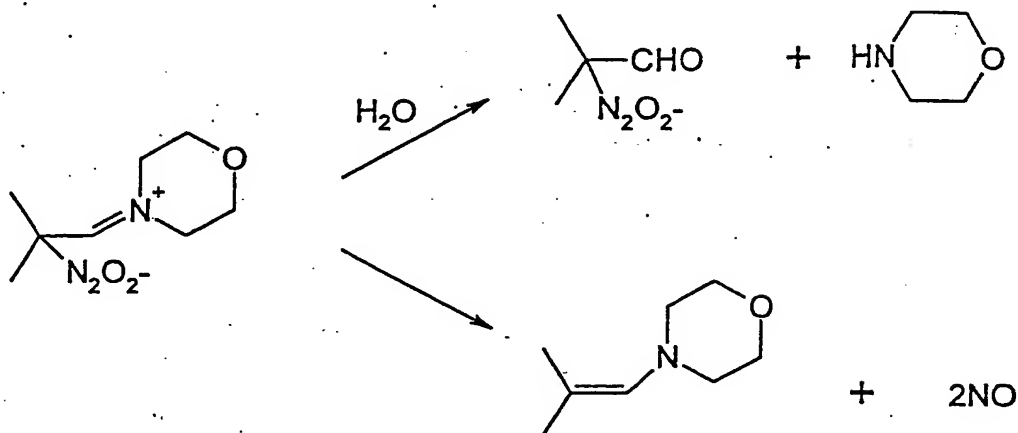
It will be appreciated by those of ordinary skill in
5   the art that either the amidine-derived or enamine-
derived diazeniumdiolates in accordance with the present
invention can be formed as a salt, and preferably, a
biologically acceptable salt. Accordingly, the
counterion is preferably any biologically acceptable
10 acceptable counterion. Such counterions can include, but
are not limited to, sodium ion, potassium ion, quaternary
ammonium ions, and the like.

Also provided by the present invention is a method
of producing a nitric oxide-releasing compound from a
15 compound containing a primary amine and/or a secondary
amine. The method comprises (a) treating the compound
containing a primary amine and/or a secondary amine with
an acetamidating agent, by which is meant an organic
chemical reagent capable of transferring the $CH_3C(=NH)^-$
20 group from itself to another molecule. Such reagents are
generally acetimidates, for example, ethyl acetimidate,
or thioimidates, for example, benzyl thioacetimidate.
The preferred reagent for use in the context of this
method is that described in Shearer et al., <u>Tetrahedron
25 Letters</u> 38(2): 179-182 (1997), so as to form an
acetamidine derivative of the compound containing the
primary amine and/or secondary amine, and (b) treating
the acetamidine derivative with nitric oxide gas to form

an amidine-derived diazeniumdiolate. This method in
accordance with the invention provides a method for
preparing an amidine-based diazeniumdiolate in which the
NO-releasing $N_2O_2^-$ functional group is bound to a carbon

5 atom rather than to the original primary or secondary
amine. In this way, many primary and secondary amine-
containing drugs can be subjected to the acetamidating
reagent to produce the amidine which can then be
converted to the diazeniumdiolate. This is advantageous

10 particularly in the case of primary amines where the N-
$N_2O_2^-$ functionality is not very stable.
As is well known in the art, nitric oxide and
compounds comprising $N_2O_2^-$ functional groups can have a
wide range of utilities, in part because of the

15 multifaceted role of nitric oxide in bioregulatory
processes. Accordingly, the present invention also
provides a composition, including a pharmaceutical
composition, comprising a present inventive
diazeniumdiolate. Preferably, the pharmaceutical

20 composition additionally comprises a pharmaceutically
acceptable carrier.
One skilled in the art will appreciate that suitable
methods of administering a diazeniumdiolate composition
of the present invention to an animal, such as a mammal,

25 are available, and, although more than one route can be
used to administer a particular composition, a particular
route can provide a more immediate and more effective
reaction than another route. Pharmaceutically acceptable
carriers are also well-known to those who are skilled in

30 the art. The choice of carrier will be determined, in
part, both by the particular composition and by the
particular method used to administer the composition.
Accordingly, there is a wide variety of suitable
formulations of the pharmaceutical compositions of the

35 present invention.
Formulations suitable for oral administration can
consist of (a) liquid solutions, such as an effective

ampules and vials, and can be stored in a freeze-dried
(lyophilized) condition requiring only the addition of
the sterile liquid carrier, for example, water, for
injections, immediately prior to use. Extemporaneous
5   injection solutions and suspensions can be prepared from
sterile powders, granules, and tablets of the kind
previously described.

The dose administered to an animal, particularly a
human, in the context of the present invention should be
10   sufficient to effect a therapeutic response in the animal
over a reasonable time frame. The dose will be
determined by the strength of the particular compositions
employed (taking into consideration, at least, the rate
of NO evolution, the extent of NO evolution, and the
15   bioactivity of any decomposition products derived from
the diazeniumdiolates) and the condition of the animal,
as well as the body weight of the animal to be treated.
The size of the dose also will be determined by the
existence, nature, and extent of any adverse side-effects
20   that might accompany the administration of a particular
composition. A suitable dosage for internal
administration is 0.01 to 100 mg/kg per day. A preferred
dosage is 0.01 to 35 mg/kg per day. A more preferred
dosage is 0.05 to 5 mg/kg per day. A suitable
25   concentration of a enamine- or amidine-derived
diazeniumdiolate in pharmaceutical compositions for
topical administration is 0.05 to 15% (by weight). A
preferred concentration is from 0.02 to 5%. A more
preferred concentration is from 0.1 to 3%.

30   In view of the above, the present invention provides
methods of using a nitric oxide-releasing amidine- or
enamine-derived diazeniumdiolate. In one embodiment, a
method of treating an animal, such as a mammal, with a
biological disorder treatable with nitric oxide, is
35   provided. The method comprises administering to the
animal, e.g., the mammal, in need thereof an amount of an
enamine- or amidine-derived diazeniumdiolate sufficient

to treat the biological disorder in the animal. In this embodiment, "biological disorder" can be any biological disorder, including hypertension, restenosis, impotency, and a biological disorder due to a genetic defect or

5   infection with an infectious agent, such as a virus, bacterium or parasite, as long as the disorder is treatable with nitric oxide.

        With regard to the above, NO- and/or NO⁻-releasing compounds derived from amidines are advantageous inasmuch

10   as amidines are present in many already approved medicinal agents, e.g., tranquilizers, α-adrenergic antagonists, like phentolamine, and nasal decongestants. Specific examples include tolazoline and diazoxide. Other examples of amidine-containing compounds include

15   methyl pyrimidine and 1,8-diamino octahydronaphthalene.

        In another embodiment of a method of use, a method is provided for treating an animal, such as a mammal, for infection with, for example, a virus, a bacterium, or a parasite. The method comprises administering to the

20   animal, e.g., the mammal, an amount of a diazeniumdiolate sufficient to treat the infection in the animal.

        In yet another embodiment, a method for treating an animal, such as a mammal, for cancer is provided. The method comprises administering to the animal, e.g., the

25   mammal, an amount of diazeniumdiolate sufficient to prevent the growth or metastasis of the cancer in the animal or to render it more susceptible to radiation or chemotherapy.

        In another embodiment, a method is provided for

30   treating an inanimate object for the presence of a potentially infectious virus, bacterium, or parasite. The method comprises contacting the inanimate object with an amount of a present inventive diazeniumdiolate sufficient to reduce the presence of the potentially

35   infectious virus, bacterium or parasite. By "potentially infectious" is meant the capability of infecting an animal, such as a mammal.

It is contemplated that the diazeniumdiolates derived from enamines and amidines in accordance with the present invention can be used to coat prostheses, stents, and medical implants, such as breast implants, prior to

5 surgical introduction into the body as a means of reducing the risk of solid state carcinogenesis associated therewith, or as a means of preventing adhesion of platelets to the implants. Additionally, the prostheses and implants can be manufactured using an

10 enamine- or amidine-derived diazeniumdiolate as an integral component of the starting materials. Medical devices incorporating an enamine- or amidine-derived diazeniumdiolate provide an invaluable two-pronged approach to the treatment of many biological disorders,

15 providing useful medical structures that also advantageously provide local release of NO.

The diazeniumdiolates derived from enamines and amidines also have utility in the *in vitro* study of NO biology.

20

EXAMPLES

The following examples further illustrate the present invention and, of course, should not be construed as in any way limiting its scope.

25   All melting points were determined on a hot stage and are uncorrected. The ^{1}H NMR spectra were determined at 200 MHz with a Varian XL-200 spectrometer and the ^{13}C NMR spectra were obtained at 50 MHz using the same instrument. The chemical shifts are expressed in δ

30 values (ppm) relative to either tetramethylsilane or sodium 3-(trimethylsilyl)propionate-d$_4$ as internal standards. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA).

Except as noted here, all reagents and amines were

35 obtained from Aldrich Chemical Company (Milwaukee, WI). Reaction solvents were Aldrich anhydrous grade but all others were reagent grade. Commercial grade nitric oxide

was obtained from Matheson Gas Products and was used as received.

Reactions under pressure were conducted in standard glass hydrogenation bottles as previously described

5 (Hrabie et al., _J. Org. Chem._ 58: 1472-1476 (1993)). The general directions are repeated here for completeness.

Given that stainless steel (SS) is required for prolonged exposure to NO gas and amines degrade most

10 types of stoppers and gaskets, a specialized reactor modeled after the standard Parr 3911 hydrogenation apparatus (Parr Instrument Co., Moline, IL) was constructed. The reservoir was replaced by a type 304 SS gas sampling cylinder equipped with SS fittings

15 (available from any "valve and fitting" plumbing supply company). The valves were diaphragm-seal packless type (Aldrich), and the pressure gauges were SS (Air Products). The usual Parr clamp and bottle system was employed but was connected to the gas reservoir via a

20 Teflon tube and mounted to allow stirring with a magnetic stirrer.

All of the analytical data given were obtained using the products as isolated directly from the reaction mixtures.

25

EXAMPLE 1

This example describes a generalized procedure for the preparation of NO- and/or NO⁻-releasing compounds from amidines.

30 A solution of the appropriate amidine, which was obtained commercially (Aldrich) or synthesized in accordance with standard procedures, in the desired solvent was placed in a standard Parr hydrogenation bottle. Nitrogen was passed through the apparatus and

35 bubbled through the solution for 5-10 min, the bottle was clamped, and NO gas was admitted to a pressure of 5 atm. The solution was stirred for the indicated time at room

temperature with addition of NO as needed during the
first 5-6 h to maintain the reservoir pressure. Excess
NO was then vented and N_2 was bubbled through the
resultant white slurry for 5 min. The product was
isolated by filtration, washed with the reaction solvent,
then washed with ether and dried *in vacuo* for several
hours. All of the products were amorphous, voluminous
white powders, which were air-stable but were stored in a
refrigerator.

EXAMPLE 2

This example describes the preparation of 2-methyl-
2-imidazoline tetrakis(nitric oxide)adduct and its sodium
salt.

A solution of 2-methyl-2-imidazoline (lysidine, 5.0
g, 59.4 mmol) in 150 ml acetonitrile was reacted with NO
for 28 h as described above. Yield 3.59 g (49%); m.p.
102-103 °C dec.; ^{1}H NMR (D_2O) δ 1.92 (6H, s), 3.51 (8H,
s), 3.67 (4H, s); ^{13}C NMR (D_2O) 24.8, 42.4, 42.5, 44.6,
51.3, 51.7, 163.5, 177.2; UV (0.01 N NaOH) λ_{max} 260 nm, =
13,600 $M^{-1}cm^{-1}$, 206 nm, ε = 22,500. Anal. Calcd for
$C_{12}H_{24}N_{10}O_4$: C, 38.71; H, 6.50; N, 37.61. Found: C, 38.92;
H, 6.55; N, 37.62.

To prepare the disodium salt, 1.74 g of a 25% NaOMe
in MeOH solution (Aldrich, 8.06 mmol) was diluted with
0.5 ml MeOH and to this was added 1.5 g of the above
diimidazolinium salt (8.06 mmol). The solid slowly
dissolved and then re-precipitated. The slurry was
diluted with acetonitrile, filtered and the solid dried
in vacuo to afford a white powder. Yield 0.92 g (92%).
m.p. >180 °C (chars); ^{1}H NMR (D_2O) δ 2.7-2.8 (2H, m),
3.3-3.4 (2H, m).

EXAMPLE 3

This example describes the preparation of
acetamidine tetrakis(nitric oxide)adduct.

A solution of acetamidine hydrochloride (7.0 g, 74.0 mmol) in 150 ml acetonitrile was treated with 16.93 ml of 25% NaOMe in MeOH (74.0 mmol) and the precipitated sodium chloride was removed by filtration. The resulting solution was treated with NO for 16 h to yield a tan powder. Yield 5.95 g (82%); m.p. >150 °C (chars); ^{1}H NMR (D_2O) δ 2.21 (s); ^{13}C NMR (D_2O) 20.8, 51.8, 57.7, 164.6.

EXAMPLE 4

This example describes the preparation of 2-iminopiperidine bis(nitric oxide)adduct.

A solution of 2-iminopiperidine hydrochloride (5.0 g, 37.2 mmol) in 200 ml acetonitrile was treated with 8.5 ml of 25% NaOMe in MeOH (37.2 mmol) and 10 ml MeOH and the precipitated sodium chloride was removed by filtration. The resulting solution was treated with NO for 23 h to yield an off-white powder. Yield 4.5 g (95%); m.p. 110-112 °C (dec.); ^{1}H NMR (D_2O) δ 1.8-1.9 (6H, m), 2.55-2.65 (2H, m), 2.85-2.95 (2H, m), 3.3-3.4 (2H, m), 3.5-3.6 (2H, m); ^{13}C NMR (D_2O) 19.0, 20.3, 23.0, 28.3, 29.0, 43.7, 44.1, 90.6, 100.5, 162.6.

EXAMPLE 5

This example describes the preparation of 2-cyclohexyl-2-imidazoline bis(nitric oxide)adduct.

The starting material for this preparation was produced by the method described by Neef et al. (J. Org. Chem. 46: 2824-2826 (1981)). A solution of 2-cyclohexyl-2-imidazoline (5.0 g, 32.8 mmol) in 300 ml acetonitrile was reacted with NO for 78 h. Yield 6.66 g (97%); m.p. 158-159 °C (dec.); ^{1}H NMR (D_2O) δ 1.4-1.7 (6H, m), 1.9-2.1 (2H, m), 2.5-2.6 (2H, m), 4.0 (4H, s); ^{13}C NMR (D_2O) 23.9 (2C), 26.6, 34.3 (2C), 47.3 (2C), 73.0, 173.4.

EXAMPLE 6

This example describes the preparation of tetrahydrozoline bis(nitric oxide)adduct.

A solution of tetrahydrozoline hydrochloride (10.0 g, 42.25 mmol) in 9.66 ml of 25% NaOMe in MeOH (42.25 mmol NaOMe) was diluted with 200 ml acetonitrile and the precipitated sodium chloride was removed by filtration. The resulting solution was treated with NO for 24 h. Yield 9.0 g (82%); m.p. 168-169 °C (dec.); ^{1}H NMR (D$_2$O) δ 1.8-1.9 (2H, m), 2.3-2.45 (1H, m), 2.9-3.0 (3H, m), 4.00 (4H, s), 7.15-7.47 (4H, m); ^{13}C NMR (D$_2$O) 20.4, 30.6, 34.7, 47.7 (2C), 76.0, 129.7, 130.7, 131.8, 133.0, 133.1, 141.5, 173.7. Anal. Calcd. for C$_{13}$H$_{16}$N$_4$O$_2$: C, 59.99; H, 6.20; N, 21.52. Found: C, 60.05; H, 6.14; N, 21.48.

EXAMPLE 7

This example describes the preparation of idazoxan-bis(nitric oxide) adduct available from Research Biochemicals, Inc. (Natick, HA).

A solution of idazoxan hydrochloride (1.00 g, 4.155 mmol) in a mixture of 0.95 ml 25% NaOMe in MeOH (4.155 mmol NaOMe) and 3 ml MeOH was diluted with 40 ml acetonitrile and the precipitated sodium chloride was removed by filtration. The resulting solution was treated with NO for 21 h. Yield 0.62 g (56%); m.p. 152-154 °C (dec.); ^{1}H NMR (D$_2$O) δ 4.04 (4H, s), 4.64 (1H, d), 5.13 (1H, d), 7.02-7.22 (4H, m). Anal. Calcd. for C$_{13}$H$_{16}$N$_4$O$_2$: C, 49.81; H, 4.94; N, 21.12. Found: C, 50.22; H, 4.61; N, 20.98.

EXAMPLE 8

This example describes a general procedure for preparation of diazeniumdiolate derivatives of enamines.

Enamines were prepared from an equimolar mixture of an aldehyde and ketone and a wide variety of secondary amines via dehydration. Such methods are described in Hicknott, Tetrahedron 38: 1975-2050, and 3363-3446 (1982); Cook, Enamines: Synthesis, Structure and Reactions, Marcel Dekker, New York (1988); and Szmuszkovicz, "Enamines", Chapter 4, In advances in Org.

Chem, Methods and Results, Wiley Interscience, New York
(1963). Preferred amines include dimethylamine,
diethylamine, piperidine, pyrrolidine, morpholine and N-
methyl-aniline.

5   These compounds were prepared according to the
general procedure set forth in Example 1, except that the
reactions were cooled when required and some gave
crystalline products as indicated in the individual
descriptions.

10

        EXAMPLE 9
        This example describes the preparation of
cyclohexanone morpholine enamine bis(nitric oxide)
adduct.

15   A solution of the enamine derived from morpholine
and cyclohexanone (15.0 g, 89.7 mmole) in 150 ml ethyl
ether was cooled in dry ice without stirring and reacted
with NO for 20 h as it warmed to room temperature.
Workup as above produced large clear crystals of product.
20 Yield 8.14 g (40%); m.p. 85-87 °C; ^{1}H NMR (CD$_3$CN) δ 1.5-
2.3 (6H, m), 2.44-2.55 (4H, m), 2.85-2.96 (4H, m), 5.13-
5.18 (1H, m), 5.23-5.27 (1H, t), 11.6 (1H, br.s); ^{13}C NMR
(CD$_3$CN) 19.2, 24.7, 28.6, 50.6 (2C), 67.1, 67.5 (2C),
112.5, 141.3; exact mass calcd. for C$_{10}$H$_{17}$N$_3$O$_3$ (M$^+$)
25 227.1269, found 227.1254. Anal. Calcd. for C$_{10}$H$_{17}$N$_3$O$_3$: C,
52.85; H, 7.54; N, 18.49. Found: C, 53.32; H, 7.63; N,
18.76.

        EXAMPLE 10
30   This example describes the preparation of
isobutyraldehyde morpholine enamine bis. (nitric oxide)
adduct.
        A solution of the enamine derived from morpholine
and isobutyraldehyde (7:0 g, 49.6 mmole) in 100 ml THF
35 was reacted with NO for 22 h as described above. Yield
4.05 g (41%); m.p. 91-92 °C; ^{1}H NMR (D$_2$O) δ 1.48 (6H, s),
3.25-3.31 (4H, m), 3.92-3.98 (4H, m), 5.26 (1H, s); ^{13}C

NMR (D_2O) 23.2 (2C), 46.1 (2C), 66.6 (2C), 75.7, 95.2; exact mass calcd. for $C_8H_{16}N_3O_3$ (MH^+) 202.1192; found 202.1137. Anal. Calcd. for $C_8H_{15}N_3O_3$: C, 47.75; H. 7.51; N, 20.88. Found: C, 47.74; H, 7.70; N, 20.13.

EXAMPLE 11

This example describes the preparation of cyclohexanecarboxaldehyde morpholine enamine bis (nitric oxide) adduct.

A solution of 4-(cyclohexylidenemethyl)morpholine (10.0g, 55.2 mmol) in 200mL of CH_3CN was cooled at 0°C in an ice bath and reacted without tirring with NO as described above for 6 h and then warmed to room temperature. The product was isolated by filtration, washed with CH_3CN, then ether and dried in vacuo. Yield 7.13g (54%); mp 115-117°C; ^1H NMR δ 1.25-1.40 (2H, m), 1.48-1.70 (4H, m), 1.95-2.40 (4H, m), 3.20-3.26 (4H, m), 3.90-3.96 (4H, m), 5.05 (1H, s); ^{13}C NMR δ 24.1 (2C), 27.8, 31.3 (2C), 46.3 (2C), 67.1 (2C), 78.0, 95.7.

Anal. Calcd for $C_{11}H_{19}N_3O_2$: C, 54.76; H, 7.94; N, 17.41. Found: C, 54.93; H, 8.04; N, 17.60.

EXAMPLE 12

This example describes the preparation of isobutyraldehyde piperidine enamine bis(nitric oxide) adduct (25).

A solution of the enamine derived from piperidine and isobutyraldehyde (5.0 g, 35.9 mmole) in 150 ml CH_3CN was stirred at room temperature and reacted with NO for 23 h as described above. Yield 3.25 g (45%); m.p. 84-85 °C; ^1H NMR (D_2O) δ 1.48 (6H, s), 1.66-1.83 (6H, m), 3.13-3.18 (4H, m), 5.25 (1H, s). ^{13}C NMR (D_2O) 23.2 (2C), 24.3, 25.1 (2C), 47.4 (2C), 75.5, 95.2. Anal. Calcd. for $C_9H_{17}N_3O_2$: C, 54.25; H, 8.60; N, 21.09. Found: C, 52.69; H, 8.56; N, 21.28.

EXAMPLE 13

This example describes the preparation of isobutyraldehyde pyrrolidine enamine bis (nitric oxide) adduct.

5   A solution of N-(2-methyl-1-propenyl)pyrrolidine (10.0g, 79.9 mmol) in 200 mL of CH_3CN was cooled to 0°C in an ice bath and reacted without stirring with NO as described above for 6 h and then warmed to room temperature. The product was isolated by filtration,
10   washed with CH_3CN, then ether and dried *in vacuo*. Yield 88.8g (60%); mp 75-76°C; ^1H NMR δ 1.48 (6H, s), 1.98-2.03 (4H, m), 3.23-3.32 (4H, m), 5.25 (1H, s); ^{13}C NMR δ 23.2 (2C), 26.5 (2C), 48.3 (2C), 75.6, 95.2.

15   EXAMPLE 14

This example describes the preparation of isobutyraldehyde N-methylaniline enamine bis(nitric oxide) adduct.

A solution of the enamine derived from N-
20   methylaniline and isobutyraldehyde (5.0 g, 31.0 mmole) in 150 ml CH_3CN was stirred at room temperature and reacted with NO for 20 h. The resulting pale yellow solution was concentrated to dryness on a rotary evaporator and the residual solid was recrystallized from absolute ethanol
25   to yield 2.26 g (33%) of product as pale, cream-colored needles. m.p. 83-84 °C; ^1H NMR (CDCl$_3$) δ 1.59 (3H, s), 1.63 (3H, s), 2.75 (3H, s), 6.00 (1H, s), 6.96-7.37 (5H, m); ^{13}C NMR (CDCl$_3$) 17.4, 26.8, 34.4, 75.6, 101.1, 118.9 (2C), 122.7, 129.4 (2C), 149.3. Anal. Calcd. for
30   $C_{11}H_{15}N_3O_2$: C, 59.71; H, 6.83; N, 18.99. Found: C, 59.77; H, 6.84; N, 19.01.

EXAMPLE 15

This example describes the preparation of
35   isobutyraldehyde N-methyl-p-toluidine enamine bis(nitric oxide) adduct.

A solution of the enamine derived from N-methyl-p-toluidine and isobutyraldehyde (5.0 g, 28.5 mmole) in 150 ml CH_3CN was stirred at room temperature and reacted with NO for 20 h. The resulting pale yellow-orange solution

5   was concentrated to dryness on a rotary evaporator and the residual off-white solid was recrystallized from absolute ethanol to yield 2.21 g (33%) of product as white cotton-like needles. m.p. 127-128 °C; ^1H NMR ($CDCl_3$) δ 1.58 (3H, s), 1.61 (3H, s), 2.31 (3H, s), 2.71

10 (3H, s), 5.92 (1H, s), 6.90-7.15 (4H, m); ^{13}C NMR ($CDCl_3$) 17.3, 20.5, 26.8, 34.9, 75.4, 101.9, 119.7 (2C), 129.9 (2C), 132.7, 147.2. Anal. Calcd. for $C_{12}H_{17}N_3O_2$: C, 61.26; H, 7.28; N, 17.86. Found: C, 61.32; H, 7.35; N, 17.88.

15 EXAMPLE 16

This example describes the preparation of isobutyraldehyde N-methyl-p-anisidine enamine bis (nitric oxide) adduct.

A solution of the enamine derived from N-methyl-p-

20 anisidine and isobutyraldehyde (5.0 g, 26.1 mmole) in 150 ml CH_3CN was stirred at room temperature and reacted with NO for 23 h. The resulting pale brown solution was concentrated to dryness on a rotary evaporator and the residual oil was crystallized from absolute ethanol to

25 yield 4.89 g (75%) of product as colorless chunky crystals. m.p. 97-98 °C; ^1H NMR ($CDCl_3$) δ 1.58 (3H, s), 1.60 (3H, s), 2.67 (3H, s); 3.79 (3H, s), 5.80 (1H, s), 6.84-7.06 (4H, m); ^{13}C NMR ($CDCl_3$) 17.2, 26.8, 36.1, 55.5, 75.2, 103.0, 114.6 (2C), 122.8 (2C), 143.4, 156.3. Anal.

30 Calcd. for $C_{12}H_{17}N_3O_3$: C, 57.35; H, 6.82; N, 16.72. Found: C, 57.36; H, 6.87; N, 16.75.

EXAMPLE 17

This example describes the measurement of the

35 production of NO and N_2O by amidine/nitric oxide adducts.

As a demonstration of the efficacy of the amidine/nitric oxide adducts described herein as nitric

oxide and nitroxyl releasing agents, selected compounds
were dissolved in either 0.1 N HCl or pH 7.4 buffer and
the headspace was monitored by chemiluminescence (to
detect NO) and gas chromatography (to detect N_2O, the
5   dehydrated dimer of HNO).   The results are shown in
Table I.

Table I

Cmpd of Ex. No.	Solution	Ratio N_2O:NO	Yield (in moles per mole cmpd)	
			N_2O	NO
2	0.1 N HCl	2:1	0.9	0.45
3	pH 7.4	13:1	0.64	0.05
4	pH 7.4	6:1	0.45	-.0.08
5	0.1 N HCl	---	0.2	N.D.*
6	pH 7.4	---	0.4	N.D.*

10

*The compounds of Examples 5 and 6 released NO too slowly
for practical measurement by headspace analysis.

EXAMPLE 18
15   This example describes the measurement of the time
course of NO production by amidine and enamine nitric
oxide adducts.
       To demonstrate the utility of these compounds as
long-term nitric oxide releasing agents, selected
20   compounds were dissolved in phosphate buffer at pH 7.4
and incubated in a 37 °C thermostated water bath.   The NO
release rate was measured periodically by flushing the

solution with inert N_2 gas and then sweeping newly generated NO into a chemiluminescence detector and integrating the signal produced over the next 4-7 mins. NO release was measured over a period of two weeks.

5   None of these compounds released nitric oxide via a single pathway which produced a release profile consistent with first order kinetics. Accordingly, the results of each test are summarized here by giving the initial NO release rate, the rate at one intermediate

10   timepoint and the total time of observed NO release for representative examples.

    Thus, the compound of Example V (tetrahydrozoline diazeniumdiolate) showed an initial NO release rate of 3.64×10^{-11} moles NO per minute per milligram of

15   dissolved sample which decreased to 2.06×10^{-11} moles NO per min. per mg. after 7 days and continued for several weeks although the last quantitative measurement showed an NO release rate of 9.00×10^{-12} moles NO per min. per mg. 15 days after the beginning of the experiment.

20   Likewise, the compound of Example VI (idazoxan diazeniumdiolate) showed an initial NO release rate of 5.25×10^{-11} moles NO/min./mg. which gradually increased to 1.41×10^{-10} moles NO/min./mg. after 4 days and then gradually decreased, reaching zero (i.e., no more NO was

25   being given off) by day 16.

    Among the enamine-derived compounds, the compound of Example VII (the diazeniumdiolate of the morpholine enamine of cyclohexanone) showed an initial NO release rate of 4.2×10^{-11} mole NO/min./mg. which decreased with

30   nearly first order kinetics to 1.8×10^{-11} mole NO/min./mg. after 3 days and reached zero by day 7.

    The enamine-derived diazeniumdiolate of Example VIII (from the morpholine enamine of isobutyraldehyde) showed an initial NO release rate of 3.7×10^{-11} mole NO/min./mg.

35   which rapidly decreased to a rate of 7.0×10^{-12} mole NO/min./mg. and then remained at about this level for 4 days before slowly declining, reaching zero after 7 days.

All publications cited herein are hereby incorporated by reference to the same extent as if each publication were individually and specifically indicated to be incorporated by reference and were set forth in its

5   entirety herein.

While this invention has been described with emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that the preferred embodiments may be varied. It is intended that the

10   invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the appended claims.

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